

Evaluation of parameters affecting quantitative detection of *Escherichia coli* O157 in enriched water samples using immunomagnetic electrochemiluminescence

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Received 6 January 2003; received in revised form 29 July 2003; accepted 29 July 2003

Abstract

We report here the use of immunomagnetic (IM) electrochemiluminescence (ECL) for quantitative detection of *Escherichia coli* O157:H7 in water samples following enrichment in minimal lactose broth (MLB). IM beads prepared in-house with four commercial anti-O157 monoclonal antibodies were compared for efficiency of cell capture. IM-ECL responses for *E. coli* O157:H7 (strain SEA13B88) were similar for all four commercial anti-O157 LPS monoclonal antibodies. The ECL signal was linearly correlated with *E. coli* O157:H7 cell concentration, indicating a constant ECL response per cell. Twenty-two strains of *E. coli* O157:H7 or O157:NM gave comparable ECL signals using IM beads prepared in-house. To assess the potential for interference from background bacteria in MLB-enriched water samples, 10^4 cells of *E. coli* O157:H7 (strain SEA13B88) were added to enriched samples prior to analysis. There was considerable variability in recovery of *E. coli* O157:H7 cells; net ECL signals ranged from 1% to 100% of expected values (i.e., percent inhibition from 0% to 99%). Cultures of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Enterobacter cloacae*, subsequently isolated from MLB-enriched water samples via IM separation (IMS), were observed to interfere with the binding of *E. coli* O157:H7 cells to IM beads. Recoveries of 10^4 *E. coli* O157:H7 cells were $\leq 10\%$ in the presence of ca. 10^8 *K. pneumoniae*, *K. oxytoca*, or *E. cloacae* cells. None of these strains gave a positive IM-ECL signal. Although competitive binding decreased sensitivity, there still was a linear correlation between ECL signal and higher *E. coli* O157:H7 cell concentrations. These studies indicate that IM-ECL in conjunction with MLB enrichment is capable of quantitatively detecting as few as 10^3 to 10^5 *E. coli* O157:H7 cells ml^{-1} , depending on percent recoveries, in enriched samples that contain ca. 10^9 total lactose-fermenting bacteria ml^{-1} . Assuming comparable growth rates for *E. coli* O157:H7 and other lactose-fermenting bacteria in MLB, it may be possible to detect as few as one *E. coli* O157:H7 in 100 ml of raw water containing as many as 10^4 to 10^6 lactose-fermenting bacteria (i.e., total coliforms).

Published by Elsevier B.V.

Keywords: Electrochemiluminescence; Enterohemorrhagic; *E. coli* O157; Immunological; Immunomagnetic separation

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1. Introduction

Enterohemorrhagic *Escherichia coli* (e.g., *E. coli* O157:H7) has emerged as a serious gastrointestinal pathogen in developed countries (Mead et al., 1999). Although the predominant mode of transmission to humans is via contaminated meat or meat products, infection from contaminated water has also been documented. One of the largest water-borne outbreaks of *E. coli* O157:H7 recently occurred in Walkerton, Ontario, Canada, with 1346 reported cases of gastroenteritis of which 167 were confirmed to be *E. coli* O157:H7 infections (Anonymous, 2000). Generic *E. coli* were detected in suspect municipal water samples; however, polymerase chain reaction (PCR) analysis was required to definitively document the presence of *E. coli* O157:H7. Ackman et al. (1997) reported an outbreak of *E. coli* O157:H7 infections (six confirmed and six probable cases) among swimmers in a fresh water lake in New York, USA. Extensive water testing was conducted within 8 days of the presumptive exposure using traditional microbiological methods, however, *E. coli* O157:H7 was not detected. These cases illustrate the difficulty in detecting water-borne enterohemorrhagic *E. coli* using traditional microbiological methods, particularly where cell numbers decrease rapidly due to dilution.

Traditional methods for detection of viable enterohemorrhagic *E. coli* rely on enrichment and plating followed by biochemical and serological characterization. Such methods are not only time-consuming and laborious, they lack sensitivity and quantitativeness. Consequently, screening of water samples for the presence of enterohemorrhagic *E. coli* (or pathogenic *E. coli* in general) is rarely conducted. Fecal coliform or generic *E. coli* populations continue to serve as pathogen surrogates or indicators, from which potential health risks are inferred.

Recently, several immunological methods have been described for the detection and enumeration of enterohemorrhagic *E. coli* O157. Most methods have been developed based on an antibody “sandwich” technique in which cells are initially captured using polyclonal or monoclonal anti-O157 antibodies conjugated to various surfaces, then subsequently labeled with a secondary antibody conjugated to a reporter

molecule. Pyle et al. (1999) described a method utilizing immunomagnetic separation (IMS) and immunofluorescent antibody (IFA) labeling followed by enumeration via solid-phase laser cytometry. Kusunoki et al. (2000) described a similar method utilizing immunolates beads in conjunction with IFA, followed by flow cytometry. Park and Durst (2000) reported an assay in which *E. coli* O157 cells were immobilized by antibodies bound to nitrocellulose and detected using immunoliposomes containing a marker dye. DeMarco et al. (1999) and Demarco and Lim (2001, 2002) have described an assay in which *E. coli* O157 cells were immobilized by antibodies bound to silica fibers, labeled with Cy-5, and an evanescent wave guide fiber-optic sensor used for quantitation. A similar method has been described where cells are immobilized by antibodies bound to glass capillary tubes, labeled with Cy-5, and an integrated waveguide fiber-optic sensor used for quantitation (Ligler et al., 2002). Yu and Bruno (1996) and Beier et al. (1998) have described assays utilizing immunomagnetic bead capture in conjunction with electrochemiluminescence (ECL) for labeling and detection of *E. coli* O157. Finally, Squirrell et al. (2002) described a method whereby cells are captured via immunomagnetic beads and lysed using specific bacteriophages, with detection using a bioluminescence assay.

Most methods appear to have been developed with the goal of rapid detection. There are many applications (e.g., food processing, clinical pathology) where rapid detection is of primary importance and results need be only qualitative (i.e., presence/absence). For example, the presence of any detectable enterohemorrhagic *E. coli* in processed food is sufficient to render that lot contaminated and unsuitable for human consumption. However, qualitative data are not adequate for purposes of water quality analysis. Quantitative data are required to establish exposure levels in recreational and potable waters for purposes of assessing public health risks. In addition, modeling of *E. coli* O157 populations in watersheds requires quantitative data to establish loading rates from runoff or fecal deposition, the effect of hydrologic parameters on transport, and the effect of various environmental parameters on mortality.

High sensitivity is also a requirement for water quality analysis. Although the infectious dose for *E. coli* O157:H7 has not been definitively established

(Kothary and Babu, 2001), epidemiological data suggest that relatively few *E. coli* O157:H7 are required for infection (Meng et al., 2001). Consequently, a detection limit of one viable cell 100 ml⁻¹ is desirable for water quality analysis.

We have previously described the application of immunomagnetic electrochemiluminescence (IM-ECL) for the quantitative detection of *E. coli* O157 in raw and concentrated water samples (Shelton and Karns, 2001). The reported maximum sensitivity of the assay was ca. 2500 *E. coli* O157 cells 100 ml⁻¹ raw water. Concentration of water samples 100-fold prior to analysis allowed for detection limits of ca. 25 cells 100 ml⁻¹. Greater concentration (ca. 1000-fold) is feasible using either larger initial water volumes and/or concentrating to smaller final volumes; however, the increased accumulation of sediments, detritus, etc., interfere with cell capture (unpublished data).

An alternative to concentration is enrichment in semi-selective broth which favors the growth of *E. coli*. The advantages of enrichment are (i) amplification of cell numbers allowing for easier detection and (ii) detection of only viable, hence potentially infectious, cells. The primary disadvantage is that, depending on the variability in bacterial growth rates between *E. coli* O157 and other bacteria in the enrichment medium, the numbers of *E. coli* O157 may be under- or over-estimated. However, *E. coli* O157 populations in raw water can still be approximated based on the relative magnitude of the IM-ECL signal in enrichment cultures. We have previously demonstrated that there is a direct correlation between IM-ECL signal and *E. coli* O157 cell numbers in surface water samples (Shelton and Karns, 2001). We report here on the evaluation of assay parameters affecting quantitative detection of *E. coli* O157 in surface water samples using IM-ECL following enrichment in a minimal lactose broth (MLB).

2. Materials and methods

2.1. Bacteria, antibodies, and reagents

E. coli O157:H7 bacterial strains used in this study are listed in Table 1. Strain SEA13B88, which was originally isolated from Odwalla unpasteurized apple

Table 1
E. coli O157 strains analyzed via IM-ECL

Strain	Serotype	Source/comments	Reference
SEA13B88	O157:H7	clinical, apple juice	Fratamico ^a
95.0080	O157:H7	clinical	ECRC ^b
96.0932	O157:H7	cow	ECRC ^b
96.0428	O157:H7	cow	ECRC ^b
96.0940	O157:H7	cow	ECRC ^b
86-24	O157:H7	clinical, HUS, strep ^R , thru mice	Griffin et al. (1988)
86-24	O157:H7	clinical, HUS	Griffin et al. (1988)
86-24	O157:H7	original, clinical, HUS	Griffin et al. (1988)
933	O157:H7	clinical, HC, original	Riley et al. (1983)
A9167-1	O157:H7	clinical, WA	CDC ^c , Karch et al. (1986)
B1375-GSC15	O157:H7	aka CDC107, clinical, NC	CDC ^c
B6550ms1	O157:H7	clinical	CDC ^c
3027-93	O157:H7	Nebraska, diarrhea	CDC ^c
3055-93	O157:H7	Louisiana, HUS	CDC ^c
C7927	O157:H7	MA, apple cider, bloody diarrhea	CDC ^c
CDC B6914-MS1	O157:H7	aka ATCC43888, clinical, feces	CDC ^d
EDL932	O157:H7	aka ATCC43894, HC, Michigan	CDC ^d
C7927	O157:H7	clinical, MA, apple cider	CDC ^d
F4546	O157:H7	clinical, MI/VA, sprouts	CDC ^d
H1827	O157:H7	clinical, CT/ILL, lettuce	CDC ^d
97A1278	O157:H7	clinical	Abbott and Janda ^d
97A2045	O157:H7	clinical	Abbott and Janda ^d
98A6049	O157:NM	clinical	Abbott and Janda ^d
H26696	O157:H7	clinical, WI, watermelon	Kehl ^c

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^e S. Kehl, Children's Hospital of Wisconsin, Milwaukee, WI.

juice, is used throughout this study as the *E. coli* O157:H7 standard strain.

Monoclonal anti-O157 antibodies were purchased from BioDesign International (BD, Saco, ME), Fitz-

gerald Industries International (FG, Concord, MA), Maine Biotechnology Services (MB, Portland, ME) and United States Biological Corporation (USB, Swampscott, MA) while a polyclonal anti-O157 antibody was purchased from Kirkegaard and Perry Labs (KPL, Gaithersburg, MD). Commercial anti-*E. coli* O157 IM beads (Dynabeads™) and streptavidin beads (2.8 µm; Dynabeads M-280™), manufactured by Dynal (Oslo, Norway), were purchased from IGEN International (Gaithersburg, MD).

MacConkey (MAC) agar plates were purchased from Spiral Biotech (Providence, RI). Minimal lactose broth (MLB) consisted of the basal salts medium of Hylemon and Phibbs (1972) (9) (50 mM potassium phosphate, 15 mM ammonium and trace nutrients) modified by the addition of 8.5 g NaCl, 1.5 g Bacto bile salts (Difco Laboratories, Detroit, MI) and 1.8 g lactose (5 mM) l⁻¹. Phosphate buffered saline (PBS-2) consisted of 150 mM potassium phosphate buffer (pH 7.2), 150 mM NaCl and 0.1% azide, while PBS-1 consisted of potassium phosphate and 150 mM NaCl at pH 7.8. Diluent consisted of PBS-2 amended with 4% (w/v) bovine serum albumin (BSA) and 1% (v/v) thesitol (polyoxyethylene 9 lauryl ether).

2.2. Bacterial isolation and culturing

Immunomagnetic separation (IMS) techniques were used to isolate bacteria bound to BD anti-O157 monoclonal antibody IM beads. A total of 10 µl of selected MLB-enriched water samples were incubated for 1 h with 40 µl of beads (ca. 10⁷ cells and 10⁷ beads) in 1 ml of PBS-2 without azide. After cell capture via IM beads, beads were shaken in PBS-2 without azide for 15 min, recaptured, and 100 µl plated onto MacConkey agar plates (two replicate plates) with an Autoplate 4000 spiral plater (Spiral Biotech). Plates were incubated overnight at 37 °C, individual colonies transferred to fresh MLB and replated on MacConkey agar plates to ensure purity. Bacteria were speciated using the BBL Enterotube II (Becton Dickinson, Sparks, MD).

E. coli O157:H7 or O157:NM strains were grown to stationary phase in 10 ml MLB overnight at 37 °C without shaking to facilitate anaerobic growth conditions. Preliminary studies indicated that *Pseudomonas* spp. were capable of rapid growth in MLB

under aerobic conditions. *E. coli* O157:H7 (strain SEA13B88) was transferred into 10 ml MLB at 1–2 week intervals for use in standard curves and inoculation experiments. Cell numbers of stationary phase *E. coli* O157:H7 (strain SEA13B88) were determined to be $9.8 \pm 0.8 \times 10^8$ ($n=6$) in a hemocytometer using phase contrast microscopy (500×).

2.3. Surface water samples and enrichment

Water samples (500 ml) were obtained from various subcatchments of the Gwynns Falls, a 17,150 ha watershed which traverses an urban–rural land-use gradient (6.7% agriculture, 18.9% forest, and 74.3% urban) in Baltimore City and Baltimore County, MD. These sites are the focus of an NSF urban Long-Term Ecological Research Project. The Gwynns Falls travels through numerous neighborhoods and public parks before draining to Baltimore Harbor and ultimately into the Chesapeake Bay. Water samples for competitive binding studies were collected on 7/22/02, enriched overnight, and analyzed on 7/24/02.

One hundred milliliters of water were filtered through a 47 mm diameter, 0.45 µm pore size sterile cellulose filter (Osmonics, Minnetonka, MN). The filters were placed into 10 ml of MLB and incubated for enrichment at 37 °C for 18–24 h without shaking to facilitate anaerobic growth conditions. Enriched samples were stored at 4 °C until analysis.

2.4. Bead capture and IM-ECL protocol

The preparation of biotinylated- and TAG-antibodies has been previously described (Shelton and Kams, 2001). IM beads were prepared in-house by incubating 2 mg of streptavidin beads for 1 h with 10 µg of biotinylated monoclonal antibody in 10 ml of diluent. Beads were harvested using a MPC-1 magnetic particle collector (Dynal), suspended in 1 ml of PBS-2 providing a 10-fold concentration, and stored at 4 °C until use.

For cell capture, the assay buffer consisted of 20 µl IM-beads, 100 µl diluent and 0.9 ml PBS-2 containing azide. For *E. coli* O157:H7 (strain SEA13B88) standard curves and pure cultures, 100 µl of culture or appropriate dilution was added to the assay buffer. For MLB-enriched water samples, 100 µl of enrichment

culture was added to the assay buffer. For inoculated MLB-enriched water samples, 100 μl of enrichment plus 100 μl of a 10^5 cells ml^{-1} *E. coli* O157:H7 (strain SEA13B88) suspension (i.e., 10^4 cells) were added to the assay buffer. After shaking for 3 h, beads were recovered for 15 min using a MPC-S magnetic particle collector (Dynal), the supernatant discarded, and beads resuspended in 0.5 ml PBS-2 and 0.1 ml diluent. To label cells, 50 μl of the TAG (ORI-TAG) antibody ($1\text{--}2\text{ }\mu\text{g ml}^{-1}$) was added and the mixture shaken for two additional hours. Final total volume was 650 μl .

2.5. IM-ECL instrumentation

Samples were analyzed using the IM-ECL instrument (ORIGEN) manufactured by IGEN International. Briefly, 550 μl of samples (85% of total volume) were pumped through a flow cell where the bead–cell–TAG complexes were magnetically captured on a platinum electrode. The sample was washed to remove contaminants and unused reagents, and a voltage was applied to create an electron transfer reaction in the presence of tripropylamine resulting in the emission of multiple photons from the Ru-chelate component of TAG. Adjustable instrument parameters were: assay gain of 100, instrument background subtracted, and signal averaged. Approximate analysis time per tube was 75 s. Net ECL units are values for spiked water samples minus values for blank water samples.

3. Results

3.1. Antibody comparisons

Standard curves of *E. coli* O157:H7 (strain SEA13B88) were run using IM beads prepared in-house with four commercial monoclonal anti-O157 antibodies (BD, FG, MB, USB) and Dynal anti-*E. coli* O157 beads. Standard curves with all four monoclonal antibodies were essentially identical with slopes of ca. 1.0 (Fig. 1); background signals varied from 100 to 200 ECL units. Dynal IM beads gave a higher signal per cell with a slope of 0.89 (Fig. 1); background ECL signals were substantially higher (ca. 2300 ECL units). Because of the lower background and slope

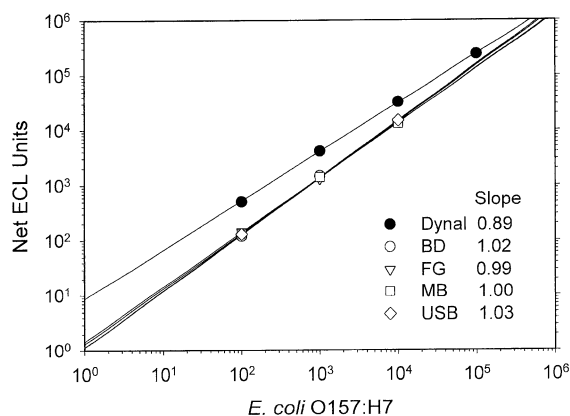


Fig. 1. Standard curves of *E. coli* O157:H7 (strain SEA13B88) in PBS-2 using commercial Dynal anti-O157 beads vs. IM beads prepared in-house with four commercial monoclonal anti-O157 antibodies: BioDesign (BD), Fitzgerald (FG), Maine Biotechnology (MB) and United States Biological (USB). Coefficients of variation were $\leq 10\%$ ($n=3$).

of ca. 1.0, indicative of a constant ECL response per cell, subsequent experiments were conducted with IM beads prepared in-house.

3.2. *E. coli* O157 strain comparisons

The 24 *E. coli* O157:H7 or O157:NM strains listed in Table 1 were analyzed by IM-ECL after growth to stationary phase in MLB (Fig. 2A). Twenty-two strains gave comparable ECL signals with ECL responses ranging from 5000 to 20,000 ECL units for ca. 10^4 cells, while two strains (96.0428 and 96.0940) gave uncharacteristically low ECL signals. When stationary phase *E. coli* O157:H7 (strain SEA13B88) cells were stored at 4 °C, ECL responses (10^4 cells) increased with culture age from ca. 8000 (day 0) to 16,000 ECL units (day 50; Fig. 2B).

3.3. Competitive binding

MLB-enriched water samples were inoculated with 10^4 *E. coli* O157:H7 (strain SEA13B88) cells to assess the affect of high background lactose-fermenting bacteria levels (ca. 10^8) on recovery and quantitation of *E. coli* O157:H7. Apparent recoveries of *E. coli* O157:H7 were highly variable (Fig. 3A). Net ECL signals ranged from 1% to 100% of expected

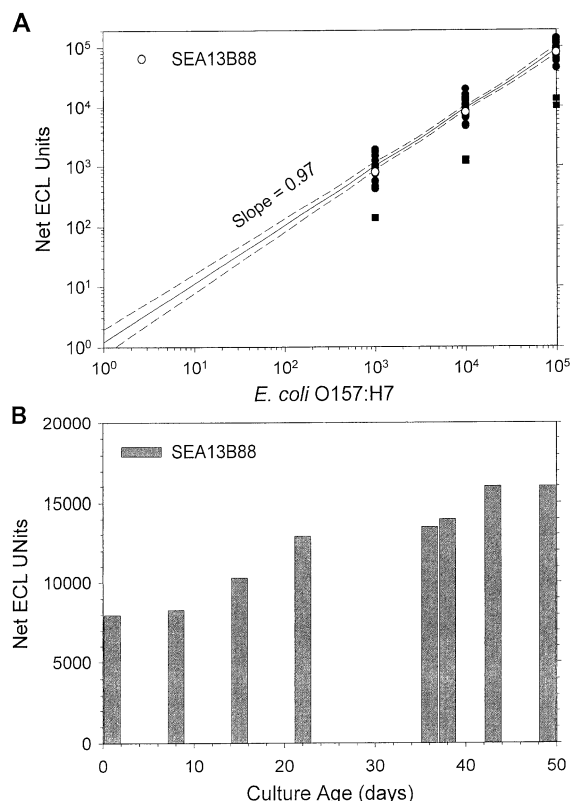


Fig. 2. (A) Standard curves with 24 *E. coli* O157:H7 or O157:NM strains using BD beads ($n=3$); open circles represent *E. coli* O157:H7 (strain SEA13B88). The solid line represents the mean slope while the dashed lines represent 95% confidence intervals; strains with square symbols were not included in calculation of mean slope or confidence intervals. (B) Affect of *E. coli* O157:H7 (strain SEA13B88) culture age on ECL signal. Coefficients of variation were $\leq 10\%$ ($n=3$).

values (i.e., percent inhibition from 0 to 99%). Selected samples were re-analyzed using the four commercial monoclonal antibodies. Although all antibodies were comparable, BD appeared to give a consistently higher response (Fig. 3B).

To assess the affect of competitive binding on *E. coli* O157:H7 quantitation, an MLB-enriched water sample which had demonstrated a high level of inhibition (sample #17, Fig. 3) was inoculated with from 10^2 to 10^6 *E. coli* O157:H7 cells. Cell numbers $<10^4$ could not be detected (Fig. 4). However, at cell numbers between 10^4 and 10^5 there was a linear ECL response; the upper dynamic range was

$<10^6$ cells (data not shown). The slope was almost identical to the *E. coli* O157:H7 standard curve in PBS-2 (Fig. 4), indicative of a constant ECL signal per cell.

Pure cultures were isolated from selected enriched water samples that demonstrated high levels of inhibition using IMS and plating. Several isolates were obtained that inhibited recoveries of 10^4 *E. coli* O157:H7 (strain SEA13B88) cells $\geq 90\%$ when present at ca. 10^8 cells (data not shown). These cultures were tentatively classified as various strains of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Entero-*

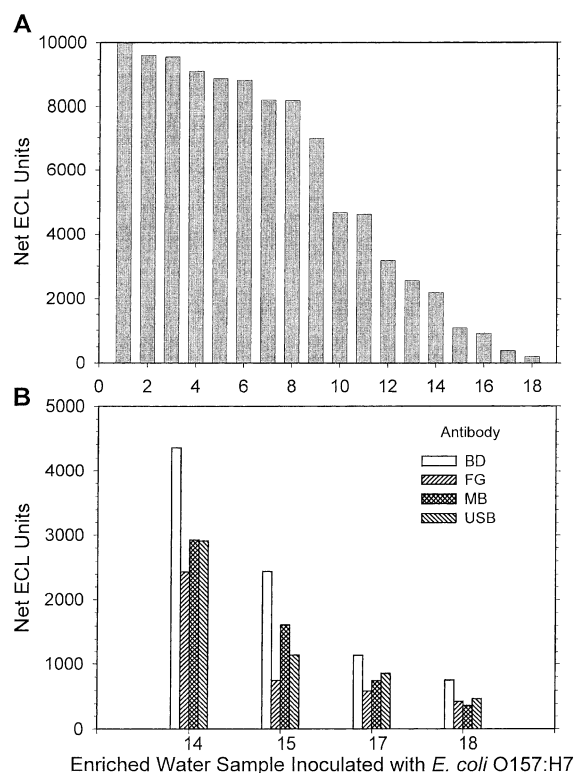


Fig. 3. (A) Recoveries of 10^4 *E. coli* O157:H7 (strain SEA13B88) cells inoculated into MLB-enriched water samples (100 μ l) containing ca. 10^8 total lactose-fermenting cells using MB beads. IM-ECL response was normalized to 10,000 ECL units = 100% recovery ($1 \text{ ECL unit cell}^{-1}$). Coefficients of variation were $\leq 30\%$ ($n=2$). (B) Comparison of *E. coli* O157:H7 (10^4 cells) recoveries using IM beads prepared in-house with four commercial anti-O157 monoclonal antibodies: BioDesign (BD), Fitzgerald (FG), Maine Biotechnology (MB) and United States Biological (USB). Coefficients of variation were $\leq 30\%$ ($n=2$).

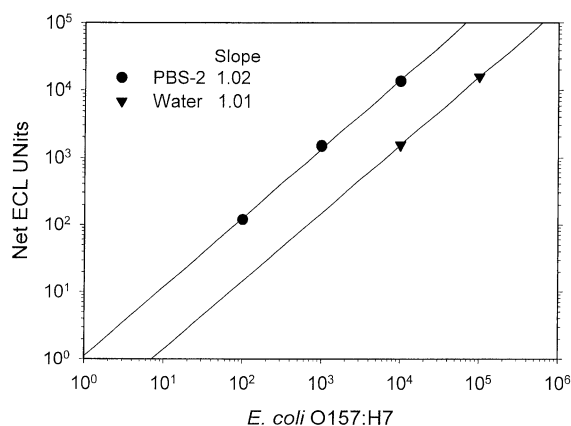


Fig. 4. Standard curves of *E. coli* O157:H7 (strain SEA13B88) using BD beads in PBS-2 vs. in an MLB-enriched water sample (#17; Fig. 3). Coefficients of variation were $\leq 30\%$ ($n=3$).

bacter cloacae. None of these strains gave a positive IM-ECL response.

4. Discussion

There is a pressing need for methods which can quantitatively and sensitively detect bacterial pathogens in recreational and potable waters. Surrogates or indicators may provide information on overall water quality, but cannot be used to accurately assess actual public health risks. Speed may be critical for certain analysis such as food processing or clinical pathology; however, for routine water analysis sensitivity and quantitation are also critical parameters. The ideal water method should be able to detect one viable cell 100 ml^{-1} raw water. To achieve this level of sensitivity with current detection methods, enrichment is required to amplify cell numbers, in conjunction with a method capable of detecting relatively small numbers of *E. coli* O157:H7 in a high background of non-O157 bacteria. In addition, the method should not give false positives (detection of non-O157 strains) or false negatives (inhibition of *E. coli* O157:H7 capture by other bacteria).

We have previously described the use of IM-ECL for quantitative detection of *E. coli* O157:H7 in raw or concentrated surface water samples. However, the reported sensitivity of 25 cells 100 ml^{-1} was inadequate for water quality analysis. Previous research

suggested that a minimal medium consisting of lactose as sole carbon source (MLB) could be used for the semi-selective growth of *E. coli* O157:H7 (Shelton and Karns, 2001).

Routine water analysis using IM-ECL in conjunction with MLB enrichment requires that *E. coli* O157:H7 strains consistently grow in MLB and give a comparable ECL response. To date, 50 strains of pathogenic *E. coli* (including 40 motile and non-motile strains of O157) have been tested and all grew on MLB at 37 °C (unpublished data). In this study, 24 *E. coli* O157 strains were tested for their IM-ECL response. Twenty-two strains gave comparable ECL signals with slopes of ca. 1.0. Some of the observed variability can be accounted for by instrument variability; we routinely observe a twofold range in ECL signal with 10^4 cells of *E. coli* O157:H7 (ca. 8000–16,000 ECL units). The remaining variability is probably due to differences in final cell concentrations at stationary phase, or cell size. It is unclear why 2 strains gave uncharacteristically low ECL signals.

The ECL signals of *E. coli* O157:H7 cultures increased as a function of culture age. This may be due to decreasing cell size with aging (Shelton and Karns, 2001). The ECL photochemical reaction only occurs within close proximity to the electrode surface. Assuming a constant number of antigen–antibody sites per cell, the total cell surface area within the reaction zone would increase as cell size decreased, enhancing the magnitude of the ECL signal.

To assess the potential for interference from background bacteria in MLB-enriched water samples, enriched samples were inoculated with 10^4 *E. coli* O157:H7 cells and the inoculated samples analyzed via IM-ECL. There was considerable variability in percent inhibition ranging from 0% to 99%. Several strains of *K. pneumoniae*, *K. oxytoca*, and *E. cloacae* were subsequently isolated via IMS which were observed to inhibit recoveries of 10^4 *E. coli* O157:H7 cells $\geq 90\%$. However, none of these strains gave a positive IM-ECL signal. It is important to note that the IM-ECL response was still linear in the presence of interfering cells, indicating that *E. coli* O157:H7 concentrations in MLB-enriched water samples can be quantified if percent binding is determined by inoculating enriched samples with a known amount of an *E. coli* O157:H7 standard.

The accuracy of immunological methods is dependent upon the fidelity of the antibody–antigen binding. The ability of monoclonal or polyclonal antibodies to bind *E. coli* O157:H7 cells, when present as pure cultures, is well documented. However, quantitative detection of *E. coli* O157:H7 in enriched water samples requires that antibodies selectively bind low numbers of *E. coli* O157:H7 cells amidst a large background of non-O157 cells. In the present study, several strains (*K. pneumoniae*, *K. oxytoca*, *E. cloacae*) were isolated from MLB-enriched water samples by IMS techniques (using the same IM beads as for IM-ECL analysis) which interfered with *E. coli* O157:H7 binding to IM beads (recoveries $\leq 10\%$). None of these strains gave a positive IM-ECL signal, indicating that attachment to anti-O157 monoclonal IM beads during the capture phase was non-specific. The IM-ECL instrument (ORIGEN) has a rigorous washing step prior to analysis, which presumably detaches the non-specifically bound cells. Binding of non-O157 bacteria to anti-O157 polyclonal IM beads has previously been reported by Porter et al. (1997). Even in those studies where IMS techniques have been successfully employed for recovery of *E. coli* O157:H7 from enrichment cultures (Elder et al., 2000), the use of a semi-selective medium (Sorbitol MacConkey agar with cefixime and tellurite; SMAC-CT) and colony morphology were required to identify presumptive *E. coli* O157:H7 colonies. It is unclear to what extent competitive binding can be mitigated through the use of more selective antibodies. Further research is required with a wider selection of antibodies to evaluate competitive binding.

In conclusion, IM-ECL allows for quantitative detection of as few as ca. 10^3 (no inhibition) to 10^5 (99% inhibition) *E. coli* O157:H7 cells ml^{-1} in MLB-enriched water samples containing ca. 10^9 total lactose-fermenting cells ml^{-1} . Determination of percent binding, based on *E. coli* O157:H7 recoveries from inoculated enrichment samples, allows for calculation of actual *E. coli* O157:H7 concentrations in MLB-enriched water samples. This information can then be used to calculate the fraction of *E. coli* O157:H7 cells in MLB-enriched water samples. For example, assuming that 10^4 *E. coli* O157:H7 cells ml^{-1} are detected in an MLB-enriched sample containing 10^9 lactose-fermenting bacteria ml^{-1} , and that percent binding is

determined to be 10%, the fraction of *E. coli* O157:H7 in the sample would be $0.0001 [(10^4/10^{-1})/10^9]$. The population of *E. coli* O157:H7 in raw water samples can then be approximated by multiplying this fraction by the population of lactose-fermenting bacteria (i.e., total coliforms) in the raw water sample. In principle, as few as one *E. coli* O157:H7 can be detected in 100 ml of raw water containing 10^4 to 10^6 lactose-fermenting bacteria (i.e., total coliforms). Studies are currently in progress to quantify the error associated with estimating *E. coli* O157:H7 populations in surface and ground water samples using MLB enrichment in conjunction with IM-ECL detection.

Acknowledgements

We thank Valerie McPhatter for technical assistance, and the hydrologic technicians of the Baltimore Ecosystem Study Long-Term Ecological Research project for collecting water samples. The use of trade names does not imply endorsement by the U.S. Department of Agriculture.

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